PERFORMANCE OF A HANDHELD PCR INSTRUMENT IN THE DETECTION OF *BACILLUS ANTHRACIS, FRANCISELLA TULARENSIS,* AND *YERSINIA PESTIS*: SENSITIVITY, SPECIFICITY, AND EFFECT OF INTERFERENTS ON ASSAY RESULTS

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ABSTRACT

Smiths Detection-Edgewood, Inc. (SDE) developed the Bio-Seeq® to provide a portable platform for use by first responders to detect biological threats in civilian areas. The Bio-Seeq® is an updated, redesigned version of a small, portable PCR instrument previously known as the HANAA, Handheld Advanced Nucleic Acid Analyzer (Higgins *et al.* 2003; Belgrader *et al.* 1998). We have tested the performance of real-time fluorogenic PCR assay reagents for the detection of three biological threat agents, *Bacillus anthracis* (BA), *Francisella tularensis* (FT), and *Yersinia pestis* (YP). All three assays are sensitive, and specific for the agents they detect. Common household substances (cornstarch, coffee creamer, baking powder, and wheat flour) interfered with the sensitivity of the assay to varying degrees that were consistent across the three assays.

1. INTRODUCTION

Each Bio-Seeq® instrument (Fig. 1) contains six thermocycler modules that are independently programmable and operable. The instrument has an LCD control panel that allows an operator to run assays, and displays the end result (in real time) of each detection reaction. Each module detects the change in the bulk fluorescence of the reaction mixture in a reaction, which indicates the presence of target DNA in the sample. The reagents are contained in a small disposable plastic device that serves as a swab for wiping a sample from a surface, a chamber for mixing the sample with buffer and the PCR reagents, and a thin, clear tube in which the PCR reaction takes place and through which the fluorescent signal is detected.

Multiple strains of BA, FT, and YP were tested to determine the ability of each assay to detect members of each species. We also tested DNA isolated from near relatives of each species, and a variety of other bacterial species to ensure that non-target organisms would not be detected by each assay. The specificity of each assay was determined by testing large amounts of total genomic DNA (100,000 copies per assay) from several non-target species of bacteria (Table 1), as well as human DNA.

Figure 1. The Bio-Seeq® instrument. Left, the lightshield is open, revealing the openings for 6 Bio-Seeq® tubes. Right, an illustration of the concept of operation. An operator, wearing protective equipment in a potentially hazardous environment, is about to conduct an assay.

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2. METHODS AND MATERIALS

2.1 Bacterial strains, culture methods, and DNA isolation

The strains used in this study were obtained from in-house culture collections at ECBC; most non-select agent strains are available from ATCC (Manassas, VA) or the Bacillus Genetic Stock Center (The Ohio State University, Columbus, OH). All strains used in this study were handled at the containment level appropriate to the organism (BSL-1, BSL-2, or BSL-3). Strains of *Y. pestis* were grown, and DNA extracted, in the ECBC BSL-3 facility. Strains of *Bacillus* spp. were grown for DNA isolation in liquid Nutrient Broth (Difco), prepared according to the manufacturer's instructions. Bacteria were grown overnight at 30°C in a shaking incubator at 220 rpm or on solid media. Cells were harvested by centrifugation, or from agar plates by suspension in sterile distilled water. DNA was extracted from bacterial cells with QIAGEN DNeasy mini spin columns and reagents (QIAGEN, Valencia, CA), using the manufacturer's instructions for the isolation of total genomic DNA. DNA was quantified and tested for purity by measuring absorbance spectrophotometrically (260/280 nm). DNA was dissolved and diluted in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

The number of PCR assay targets in target organisms was estimated to be one per genome copy. The number of copies of bacterial genomes per unit mass was calculated from reported genome sizes. Relatively few genomes have been sequenced for any given species; when available, we used the genome sizes of strains that have been sequenced as an estimate (http://www.ncbi.nlm.nih.gov/genomes/MICROBES/Complete.html). For purposes of this study, genome sizes for species without good published size information were assumed to be that of the closest sequenced neighbor.

2.2. PCR assays

The result of a PCR assay in the BioSeeq is reported as a Ct value, which is the number of PCR reaction cycles that elapse before the bulk fluorescence of the reaction mixture becomes significantly higher than the background fluorescence of the mixture before the reaction begins. The greater the amount of target DNA present, the more fluorescent dye liberated each PCR reaction cycle, and the fewer cycles required to increase the fluorescence of the reaction mixture above background. This phenomenon can be observed in the experiments presented here in which the limits of detection for each assay were determined (see below). No value is reported for negative results.

To test the specificity and inclusivity of the probe and primer sets using isolated total genomic DNA or cells/spores, samples were prepared as follows. For two reactions, a single dried bead containing the appropriate probe, primers, and internal control reagents was placed in a 1.5 ml microcentrifuge tube with two Ready-to-Go PCR reagent beads (containing *Taq* polymerase, dNTPs, and buffer) (Amersham Biosciences, Piscataway, NJ) and 48 µl nuclease-free water. When the reagent beads were dissolved, the mixture was divided into two 24 µl aliquots. One µl of DNA or cell/spore suspension was added to each reaction mixture (total volume 25 µl). Each reaction mixture was transferred to a Bio-Seeq® reaction tube, capped and covered with Parafilm, and gently centrifuged to draw the reaction mixture into the tube. Each tube was then placed into a separate PCR module in the Bio-Seeq® instrument and the thermocycling was started according to the manufacturer's instructions.

3. RESULTS

3.1 Specificity of the assay for *Bacillus anthracis.*

The assay for *B. anthracis* detects the presence of sequences specific to *B. anthracis* (not described here for proprietary reasons). Strains containing this sequence therefore should be detected by the assay. The *B. anthracis* strains used in this study that contain the target sequence are NNR-1 and ANR-1. All other strains listed are known not to contain the target sequence (due to intentional deletions of the target sequence) and were used as negative controls. Total genomic DNA was prepared from each species and strain listed in Tables 1 and 2, as described above. To determine whether the assay accurately identified *B. anthracis* strains containing the target sequence, we diluted preparations of genomic DNA from strains NNR-1 and ANR-1 to a concentration of 1000 genome copies per microliter. One microliter of sample was added to 24 µl of assay reaction mixture, placed in a reaction tube and inserted into the instrument. As positive controls, we also assembled reactions containing 1000 copies of the synthetic target DNA designed to match the assay reagents.

When the PCR thermocycler profile was run for 50 cycles, the assay detected 1000 copies of genomic DNA containing target sequences (from both strains ANR-1 and NNR-1), as well as 1000 copies of the synthetic target (Table 1). The specificity of the assay was also tested by including large amounts of total genomic DNA from *Bacillus anthracis* strains known not to contain the target DNA sequence, and from other species of *Bacillus* (Table 2). None of the other strains tested reacted positively. (Examination of the data later revealed that a single positive reaction with *B. anthracis* strain ΔSterne was attributed to the presence of an air bubble in the reaction tube during the assay).

Table 1. Detection of DNA from target-containing strains of *B. anthracis*.

Target DNA[a]	# positive/total[b]	Ct values[c]
ANR-1	3/3	32, 32, 32
NNR-1	3/3	35, 34, 34
synthetic target	2/2	33, 34
NTC	0/3	N/A

[a]1000 copies per reaction. NTC, no-template control (negative control).
[b]reactions run on either of two instruments
[c]N/A, not applicable. Values are reported only for positive assay results.

Table 2. Detection of DNA from strains of *B. anthracis* and other species, not containing the target sequence.

Target DNA[a]	# positive/total[b]	Ct values[c]
B. anthracis:		
ΔAmes	0/3	N/A
ΔSterne	1/3	47
ΔNH-1	0/3	N/A
VNR1-Δ1	0/3	N/A
NNR1-Δ1	0/3	N/A
B. thuringiensis		
4A2	0/3	N/A
4E3	0/3	N/A
4F4	0/3	N/A
405	0/3	N/A
401	0/3	N/A
4C2	0/3	N/A
4J4	0/3	N/A
4T4	0/3	N/A
ATCC 10792	0/3	N/A
var. kurstaki	0/3	N/A
B. cereus		
ATCC 11778	0/3	N/A
ATCC 13824	0/3	N/A
ATCC 6464	0/3	N/A
ATCC 12826	0/3	N/A
6E1	0/3	N/A
1122	0/3	N/A
1219	0/3	N/A
631	0/3	N/A
B. subtilis		
ATCC 23059	0/3	N/A
3A8	0/3	N/A
YB886	0/3	N/A
var. niger (*B. globigii*)	0/3	N/A
1031	0/3	N/A
B. mycoides ATCC 6462	0/3	N/A
B. megaterium ATCC 14581	0/3	N/A
B. sphaericus	0/3	N/A
synthetic target	20/20	7 @ 33, 11 @ 34, 2 @ 35
NTC	0/3	N/A

[a]100,000 copies per reaction, except for synthetic target (1000 copies). NTC, no-template control (negative control).
[b]reactions run on either of two instruments
[c]N/A, not applicable. Values are reported only for positive assay results.

3.2 Limits of detection for *Bacillus anthracis*

After observing that NNR-1 and ANR-1 genomic DNA gave positive results with the assay, we grew spores of NNR-1 for use as the target strain for limit of detection (LOD) experiments.

Assays for the detection of NNR-1 spores, using the reagent bead set were prepared as described in Materials and Methods (preparation of the Master Mix with the lyophilized reagent beads was not described in the Materials and Methods – please include). A suspension of spores was serially diluted in sterile 10 mM Tris HCl, pH 7.4 (Sigma Co., St. Louis, MO), and aliquots containing spores (or buffer alone) were added to PCR reaction mixtures prepared in microcentrifuge tubes, then placed in Bio-Seeq® reaction tubes. The reagents for the detection of *B. anthracis* allowed the detection of as few as 10^2 spores per assay (Table 3). The limit of detection, therefore, was between 10 and 100 spores in this experiment.

Table 3. Detection of NNR-1 spores using the BA reagent bead set[a].

# of spores added	# positive/total[b]	Ct values[c]
B. anthracis NNR-1		
4.5×10^5	3/3	24, 25, 25
10^5	3/3	26, 27, 28
10^4	3/3	29, 30, 31
10^3	3/3	33, 33, 34
10^2	3/3	36, 44, 48
10^1	0/3	N/A
10^0	0/3	N/A
0	0/3	N/A

[a]Reaction mixtures prepared in a microcentrifuge tube and placed directly into a Bio-Seeq® reaction tube.

[b]reactions run on either of two instruments.

[c]N/A, not applicable. Values are reported only for positive assay results.

3.3 Specificity of the assay for *Francisella tularensis*

The real-time fluorogenic PCR assay for *F. tularensis* was tested on the instrument. The assay for *F. tularensis* detects the presence of a sequence on the bacterial chromosome. Total genomic DNA was obtained from our in-house DNA collection or prepared from each species and strain tested as described above. To determine whether the assay accurately identified *F. tularensis* strains, we diluted preparations of genomic

DNA from several strains to a concentration of 1000 genome copies per microliter. One microliter of each DNA suspension was added to 24 µl of reaction mixture as described above, placed in a reaction tube and inserted into the instrument. As positive controls, we also assembled reactions containing 1000 copies of synthetic target DNA designed to match the assay reagents.

When prepared as described above, the assay detected 1000 copies of genomic DNA from several strains of *F. tularensis*, as well as 1000 copies of the synthetic target (Table 4). The specificity of the assay was also determined by testing large amounts of total genomic DNA (100,000 copies per assay) from several other species of bacteria, as well as human DNA (Table 4). No genomic DNA from the other species tested reacted positively in the *F. tularensis* assay. (Inspection of the data from the positive results in one assay for *C. perfringens* revealed that this result was likely caused by an air bubble in the reaction tube.

3.4 Limits of detection using purified genomic DNA from *F. tularensis* strain LVS.

We determined the limit of detection for *F. tularensis* strain LVS DNA after noting that it reacted positively in the assay (Table 4). *F. tularensis* strain LVS genomic DNA was diluted serially in TE buffer, and aliquots of each dilution were added to a reaction mixture as described above. As few as 62.5 copies of genomic DNA were consistently detected (Table 5). In the case of the single negative result when 50 copies of the genome were added to the assay, the trend in the data suggested that a positive result would have been reported in another few cycles (data not shown); if it had, the limit of detection in this study would have been reported as low as 30 copies per reaction.

3.5 Limits of detection using cells of *F. tularensis* strain LVS.

Assays for the detection of *F. tularensis* strain LVS cells were prepared as described above. A suspension of cells was serially diluted in sterile 10 mM Tris HCl, pH 7.4 (Sigma Co., St. Louis, MO), and aliquots containing cells (or buffer alone) were added to PCR reaction mixtures prepared in microcentrifuge tubes, then placed in Bio-Seeq® reaction tubes. The reagent bead set, prepared as described, allowed the detection of as few as 1 cell per assay (Table 6). The cause of the two positive results in the negative control tubes is unknown, but conceivably may be due to operator error or contamination.

Table 4. Specificity of reagents for *F. tularensis* tested against genomic DNA.

Source of target DNA[a]	# of copies	# positive/total[a]	Ct values[b]
F. tularensis			
LVS	1000	3/3	34, 35, 35
var. novicida	1000	6/6	31, 33, 33, 34, 35, 35
Schu 4	1000	3/3	32, 32, 33
88R675	1000	3/3	37, 37, 38
A91-1623	1000	3/3	36, 37, 37
88R144	1000	3/3	32, 32, 34
Salk	1000	3/3	30, 36, 36
Bacillus cereus	10^5	0/3	N/A
Bacillus subtilis	10^5	0/5	N/A
Bacteroides fragilis	10^5	0/3	N/A
Bordetella pertussis	10^5	0/3	N/A
Campylobacter jejuni	10^5	0/3	N/A
Clostridium perfringens	10^5	1/5	50
Clostridium tetani	10^5	0/3	N/A
Escherichia coli 43895	10^5	0/3	N/A
E. coli 0157:H7	10^5	0/3	N/A
Neisseria meningitidis	10^5	0/3	N/A
Pseudomonas aeruginosa	10^5	0/3	N/A
Salmonella typhimurium LT2	10^5	0/3	N/A
Staphylococcus aureus 14458	10^5	0/3	N/A
Streptococcus pyogenes	10^5	0/3	N/A
Homo sapiens	10^5	0/3	N/A
synthetic target	1000	17/17	30, 35, 37, 6@38, 6@39, 41, 48
No template control	0	0/9	N/A

[a]reactions run on either of two instruments. Data are combined from seven sets of experiments.
[b]N/A, not applicable. Values are reported only for positive assay results.

Table 5. Detection of *F. tularensis* strain LVS genomic DNA using FT reagent beads [a].

# of genome copies	# positive/total[b]	Ct values[c]
1000	5/5	35, 35, 35, 35, 36
750	5/5	35, 35, 36, 36, 36
500	5/5	36, 37, 39, 41, 45
250	4/4	27, 38, 38, 38
125	6/6	39, 39, 40, 41, 41, 41
62.5	5/5	39, 39, 40, 40, 42
50	4/5	41, 41, 41, 41
40	5/5	40, 44, 46, 48, 48
30	5/5	38, 41, 42, 46, 48
20	3/8	45, 47, 49
15	6/8	43, 44, 46, 46, 48, 49
10	3/10	40, 45, 46
5	1/5	49
1	0/5	N/A
0 (buffer only)	0/10	N/A
Synthetic target (1000 copies)	9/10	37, 4@38, 2@40, 41, 45

[a]Results are combined from three experiments.

[b]reactions run on either of two instruments.

[c]N/A, not applicable. Values are reported only for positive assay results.

Table 6. Detection of *F. tularensis* strain LVS cells using FT reagent beads [a].

# of colony-forming units	# positive/total[b]	Ct values[c]
10^6	11/11	5@18, 4@19, 2@20
10^5	11/11	18, 20, 21, 3@22, 4@23, 25
10^4	11/11	2@25, 4@26, 5@27
5×10^3	11/11	7@27, 4@28
10^3	11/11	27, 4@30, 5@31, 32
10^2	11/11	32, 33, 2@34, 4@35, 2@36, 37
10^1	11/11	8@38, 3@39
1 cfu	11/11	2@41, 42, 2@44, 3@45, 2@47, 49
0 (buffer alone)	2/11	45, 46
P. agglomerans (10^5 cells)	0/3	N/A

[a]Results are combined from three experiments.

[b]reactions run on either of two instruments.

[c]N/A, not applicable. Values are reported only for positive assay results.

Table 7. Detection of genomic DNA from *Y. pestis* strains [a].

Yersinia pestis strain	# of copies	# positive/total[a]	Ct values[b]
EV-76	1000	3/3	33, 33, 33
9910463	1000	3/3	33, 34, 36
9910265	1000	3/3	33, 33, 33
9910117	1000	3/3	33, 34, 34
9906414	1000	3/3	34, 33, 33
9808960	1000	3/3	33, 34, 33
9808780	1000	3/3	33, 33, 33
9808723	1000	3/3	34, 34, 34
9800419	1000	3/3	34, 33, 34
9709959	1000	3/3	33, 33, 33
97076011	1000	3/3	33, 34, 33
9168786	1000	3/3	34, 35, 34
9168781	1000	3/3	34, 34, 35
910810	1000	3/3	33, 33, 33
A294172	1000	3/3	34, 34, 34
16486	1000	3/3	33, 33, 33
A1122	1000	3/3	33, 34, 34
Amal	1000	3/3	32, 32, 33
Harbin	1000	3/3	33, 34, 34
Penn2	1000	0/3	N/A
1866	1000	0/3	N/A
Synthetic target	1000	6/7	35, 35, 35, 37, 38, 45
No template control	0	0/3	N/A

[a]reactions run on either of two instruments. Data are combined from several experiments.

[b]N/A, not applicable. Values are reported only for p ositive assay results.

Table 8. Specificity of YP assay reagents tested against non-target genomic DNA.

Source of target DNA[a]	# of copies	# positive/total[a]	Ct values[b]
Yersinia enterocolitica	10^5	0/3	N/A
Yersinia rohdei	10^5	0/3	N/A
F. tularensis Schu 4	10^5	0/3	N/A
Pantoea agglomerans	10^5	0/3	N/A
Bacillus anthracis plasmid pXO1	10^5	0/3	N/A
Bacillus cereus ATCC 14579	10^5	0/3	N/A
Bordetella pertussis ATCC 9797	10^5	0/3	N/A
Campylobacter jejuni ATCC 33560	10^5	0/3	N/A
Clostridium perfringens	10^5	0/3	N/A
Escherichia coli ATCC 43895	10^5	0/3	N/A
E. coli 0157:H7	10^5	0/3	N/A
Neisseria meningitides	10^5	0/3	N/A
Pseudomonas aeruginosa PAO1	10^5	0/3	N/A
Salmonella typhimurium LT2	10^5	1/3	50
Staph. aureus ATCC 14458	10^5	0/3	N/A
Streptococcus pyogenes	10^5	0/3	N/A
Homo sapiens	10^5	0/3	N/A
synthetic target	1000	6/6	33, 36, 36, 36, 37, 46
No template control	0	0/3	N/A

[a]reactions run on either of two instruments. Data are combined from seven sets of experiments.

[b]N/A, not applicable. Values are reported only for positive assay results.

3.6 Specificity of reagents for *Yersinia pestis* DNA

Samples of purified genomic DNA from several strains of *Y. pestis* were obtained from bacteria grown in the BL-3 laboratory, Edgewood Chemical Biological Center, prepared from each strain as described above. . Other samples were obtained from our in-house DNA collection. To determine whether the assay accurately identified *Y. pestis* strains, we diluted preparations of genomic DNA from each strain to a concentration of 1000 genome copies per microliter. One microliter of each DNA suspension was added to 24 µl of reaction mixture as described above, placed in a reaction tube and inserted into the instrument. As positive controls, we also assembled reactions containing 1000 copies of synthetic target DNA designed to match the assay reagents.

When prepared as described above, the assay detected 1000 copies of genomic DNA from several strains of *Y. pestis*, as well as 1000 copies of the synthetic target (Table 7). All *Y. pestis* strains tested were detected by the assay except strains Penn2 and 1866. The reason for this is not clear at present; however, we suspect that the target sequence recognized by this assay is not present in either of these strains. The specificity of the assay was also confirmed by testing large amounts of total genomic DNA (100,000 copies per assay) from several non-target species of bacteria, as well as human DNA. No genomic DNA from the other species tested, including strains of two other *Yersinia* species, reacted positively in the *Y. pestis* assay (Table 8). The false positive result for one test using *Salmonella typhimurium* DNA gave a C_T of 50, perhaps due to an air bubble in the reaction tube.

3.7 Limits of detection of the assay for *Y. pestis.*

The YP assay under laboratory conditions proved to be exquisitely sensitive for both *Y. pestis* genomic DNA (limit of detection = 125 copies; Table 9) and cells (limit of detection = 100; Table 10). There was good agreement between the limit of detection experiments for both cells and DNA. We noted that the C_T values obtained for 1000 copies of most *Y. pestis* targets (approximately 33; Tables 7 and 10) is consistent with the value obtained with 1000 cells of EV-76 in the dilution experiment for sensitivity (C_T=33; Table 9).

3.8 Effect of interferents on the three assays

Four common materials (innocuous "white powders") were tested for their ability to interfere with the three assays: coffee creamer (Domino Non-Dairy Creamer), baking powder (Rumford brand, Clabber Girl Inc., Terre Haute IN), wheat flour (America's Choice unbleached), and cornstarch (Giant brand). Each material is found in

Table 9. Detection of *Y. pestis* strain EV-76 genomic DNA using YP reagent beads [a].

# of genome copies	# positive/total[b]	Ct values[c]
10000	4/4	30, 30, 30, 30
1000	5/5	28, 33, 33, 33, 33
750	5/5	31, 33, 33, 33, 34
500	5/5	29. 34, 35, 35, 35
250	5/5	34, 35, 35, 36, 36
125	6/6	34, 36, 36, 36, 45
62	2/5	30, 50
50	3/5	31, 40, 47
40	4/5	42, 47, 50, 50
30	1/5	44
20	0/3	N/A
0 (buffer alone)	0/4	N/A
Synthetic target (1000 copies)	2/2	36, 36

[a]Results are combined from two experiments.

[b]reactions run on either of two instruments.

[c]N/A, not applicable. Values are reported only for positive assay results.

Table 10. Detection of *Y. pestis* strain EV-76 cells using YP reagent beads [a].

# of colony-forming units	# positive/total[b]	Ct values[c]
5×10^7	5/5	18, 18, 19, 20, 31
10^6	11/11	2, 18, 21, 5@22, 2@23, 24
10^5	11/11	22, 2@25, 7@26, 27
10^4	11/11	25, 27, 2@28, 5@29, 2@30
5×10^3	11/11	26, 2@29, 6@30, 2@31
10^3	11/11	29, 6@32, 3@33, 34
10^2	11/11	19, 30, 31, 34, 5@35, 36, 38
10^1	10/11	4@37, 3@38, 39, 45, 46
1 cfu	0/11	N/A
0.1 cfu[d]	0/11	N/A
Synthetic target (1000 copies)	10/10	32, 2@35, 6@36, 37

[a]Results are combined from four experiments.

[b]reactions run on either of two instruments.

[c]N/A, not applicable. Values are reported only for positive assay results.

[d]A further 10-fold dilution of the previous sample.

most home kitchens and in many workplaces (especially coffee creamer). They may either be found on surfaces that have been contaminated with a potential powdered biological weapon, or used by the perpetrator of a hoax, as it is widely believed that weaponized spores have the appearance of a white powder. All interferents were obtained at local supermarkets. Aliquots of each interferent were weighed and placed into 15 ml disposable plastic tubes before being supplied blind to the persons conducting the experiment. Interferents were dissolved or suspended in nuclease-free, molecular biology grade water (Gibco BRL / Invitrogen, Carlsbad, CA) before use. Tris diluent was used in place of spores in all of the negative controls.

Cornstarch had little effect on the performance of the assays (data not shown). In this study, we did not reach a concentration of cornstarch that was completely inhibitory to the assay (greater than 7.5 mg per assay). Similarly, coffee creamer, of all four of the interferents, had the least effect on the performance of the assay (data not shown). The minimum inhibitory concentration of coffee creamer in this study was in excess of 15 mg per assay.

However, both flour and baking powder inhibited the assay in quantities far less than coffee creamer or cornstarch. Amounts of flour less than 1 mg significantly inhibited the assays (data not shown). This inhibition is probably due, at least in part, to the presence of high molecular weight polysaccharides (mostly starches, in white flour) and protein (gluten). Both of these components contribute to the tendency of flour to form a sticky suspension at higher concentrations (eventually approaching the consistency of dough). Even smaller amounts of baking powder were inhibitory to the assay (data not shown). We hypothesize that the inhibition by baking powder is the result of some combination of changes in the pH of the reaction solution brought about by the acid-base reaction of baking powder components with water and generation of CO_2 by the same reaction. Another component of some baking powders (including that used in this work), calcium acid phosphate ($Ca(H_2PO_4)_2$) contains pyrophosphate, which may also inhibit *Taq* polymerase because it is a product of the DNA polymerization reaction. Some flours ("self-rising" flour) contain baking powder; the effect of these two interferents combined in this fashion was not examined in this study.

CONCLUSIONS

The Bio-Seeq® instrument and reagents correctly identified *B. anthracis*, *F. tularensis*, and *Y. pestis* strains in all cases where strains were known to contain the targeted DNA sequences. The assays did not significantly cross-react with over 30 other species and strains of bacteria, including many species in the same genus (in the

cases of *Bacillus* and *Yersinia*). Under laboratory conditions, the sensitivity of the assays was observed to be 100 spores/cells or less, depending on the assay. The addition of coffee creamer or cornstarch had little effect on the performance of the assays; however, both flour and baking powder adversely affected assay performance.

We noted that, in testing the assay for *F. tularensis*, a comparison of the LOD for genome copies (purified DNA) versus the results for cells; the LOD for purified DNA was some 30-100 times higher than the LOD for cells. This counterintuitive result suggests that either the purified genomic DNA was partially degraded or that some target sequences being detected were present in DNA clinging to the outside of cells, or carried along in the cell preparation from the initial isolation, since no steps were taken to break open cells to release the DNA contained inside. Further sample processing (resulting in recovery of most of the sample DNA in pure form) should increase the accuracy of the assay, as well as remove many of the problems caused by the presence of interferents.

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